ab107923

Glucose 6 Phosphate Assay Kit (High Sensitivity)

Instructions for Use

For the rapid, sensitive and accurate measurement of Glucose 6 Phosphate levels in various samples

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Version 3a, Last Updated 7 June 2023

Table of Contents

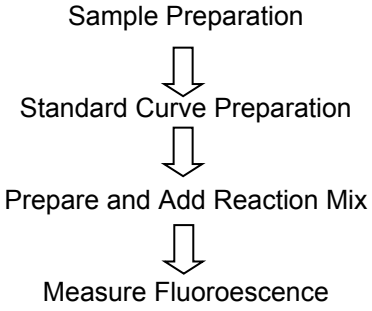
1. Overview

Glucose-6-phosphate (G6P) is a key intermediate for glucose transport into cells, which then enters either metabolic pathways or storage. G6P can enter the glycolytic pathway, the pentose phosphate shunt or be stored as glycogen or starch. G6P is utilized by its dehydrogenase to generate reducing equivalents in the form of NAD(P)H. This is particularly important in red blood cells where a G6PDH deficiency leads to hemolytic anemia.

Abcam's Glucose 6 Phosphate Assay Kit (High Sensitivity) is a simple, sensitive and rapid means of quantifying G6P in a variety of samples. In the assay, glucose-6-phosphate is oxidized with the generation of a product that converts a nearly colorless probe to an intensely fluorescent product (Ex/Em 535/587 nm).

The Glucose-6-Phosphate Assay Kit can detect G6P in the range of 10 to 500 pmoles which is equivalent to the range of 1-500 µM in the original sample assuming a dilution of 5X during processing. This High Sensitivity Glucose-6-Phosphate Assay Kit is much more sensitive than the Glucose-6-Phosphate Assay Kit (ab83426).

2. Protocol Summary



3. Components and Storage

A. Kit Components

Item	Quantity
Assay Buffer V/G6P Assay Buffer	25 mL
PicoProbe I/PicoProbe™ (in DMSO)	0.4 mL
Development Enzyme Mix IX/G6P Enzyme Mix (Lyophilized)	1 vial
Developer IX/G6P Substrate Mix (Lyophilized)	1 vial

G6P Standard/G6P Standard (10 µmol; Lyophilized)	1 vial

* Store kit at -20°C, protect from light. Warm Assay Buffer V/G6P Assay Buffer to room temperature before use. Briefly centrifuge all small vials prior to opening. Keep enzyme mix on ice while in use.

PicoProbe I/PicoProbe™: DMSO solution. Ready to use as supplied. Warm up to room temperature to thaw before using. Store at -20°C.

G6P STANDARD: Dissolve in 100 µL dH_2O to generate 100 mM (100 nmol/µL) G6P Standard solution. Keep cold while in use. Store at -20°C.

DEVELOPER IX/G6P SUBSTRATE MIX, DEVELOPMENT ENZYME MIX IX/G6P ENZYME MIX: Dissolve with 220 µL of Assay Buffer. Pipette up and down to dissolve. Aliquot into portions and store at -20°C. Avoid repeated freeze/thaw cycles. Use within two months.

B. Additional Materials Required

- PBS
- Liquid nitrogen or methanol/dry ice
- Perchloric acid 1 N
- $KHCO_3$ 3 M

- Microcentrifuge
- Pipettes and pipette tips
- Fluorescent microplate reader
- White microplate
- Orbital shaker

4. Assay Protocol

Note: White plates enhance the sensitivity of fluorescent assays and are highly recommended

1. Sample Preparation:

Liquid or solution samples can be assayed directly. For tissue or cell samples: 10-100 mg tissue or 5 million cells should be rapidly homogenized with 2-3 volumes of ice cold PBS or other buffer (pH 6.5-8). Centrifuge at top speed for 2-3 min to remove insoluble materials.

Note:

Enzymes in samples may interfere with the assay. We suggest deproteinizing samples using 10 kDa molecular weight cut off spin columns (ab93349) or by using a perchloric acid/KOH protocol as follows:

a) Tissue samples (10-100 mg) should be frozen rapidly (liquid N_2 or methanol/dry ice), weighed and pulverized.
b) Add 2 µL 1 N perchloric acid/mg per sample. KEEP COLD!
c) Homogenize or sonicate thoroughly. Spin homogenate at 10,000 x g for 5-10 minutes.
d) Neutralize supernatant with 3 M $KHCO_3$, adding repeated 1 µL aliquots/10 µL of supernatant while vortexing. Add until bubble evolution ceases (2-5 aliquots). Put on ice for 5 minutes.
e) Check pH (using 1 µL) is ~6-8. Centrifuge 2 minutes at 10,000 x g to pellet $KClO_4$.
f) Neutralize with 10 N KOH to minimize G6P conversion.

Add 1-50 µL samples into duplicate wells of a 96-well plate and bring volume to 50 µL with Assay Buffer.

For unknown samples, we suggest testing several doses of your samples to ensure readings are within the standard curve range.

Note:
NADH or NADPH in samples will generate background readings. If NADH or NADPH is in your sample, you may do a background control (omit Development Enzyme Mix IX/G6P Enzyme Mix from the reaction mix) to read the background, then subtracted the background from G6P readings.

2. Standard Curve Preparation:

Depending on your sample concentration, dilute the G6P Standard to 1 nmol/µL (1 mM) by adding 10 µL of the 100 nmol/µL Standard to 990 µL of dH_2O, mix well.

Dilute the 1 nmol/µL standard to 10 pmol/µL by adding 10 µL to 990 µL of dH_2O. Add 0, 1, 2, 3, 4, 5 µL (for 0-50 pmol range) or 0, 10, 20, 30, 40, 50 µL (for 0-500 pmol range) into a series of wells on a 96 well plate.

Adjust volume to 50 µL/well with Assay Buffer to generate 0, 10, 20, 30, 40, 50 pmol/well or 0, 100, 200, 300, 400, 500 pmol/well of G6P Standard (see standard curve below).

3. Reaction Mix: Mix enough reaction mix for the number of samples and standards to be performed: For each well, prepare a total 50 µL Reaction Mix containing:

	Reaction Mix	**Background**
Assay Buffer V/G6P Assay Buffer	42 µL	44 µL
PicoProbe I/PicoProbe™*	4 µL	4 µL
Development Enzyme Mix IX/G6P Enzyme	2 µL	---

Mix Developer IX/G6P Substrate Mix	2 µL	2 µL

Add 50 µL of the Reaction Mix to each well containing the G6P Standard and samples. Add 50 µL of the background mix into background control wells.

*** Note:** For samples containing less than 250 picomoles of G6P, reduce the probe volume to 1 µL per well to reduce reagent background and increase the reaction buffer appropriately.

4. Incubate for 5 min at room temperature, protect from light.

5. Measure fluorescence using Ex/Em = 535/587 nm with a plate reader.

5. Data Analysis

Correct reagents background by subtracting the value of the zero G6P blank from all readings. If sample background reading is significant, subtract the sample background reading from sample reading.

Plot the standard curve. Apply the corrected sample readings to the standard curve to get G6P amount in the sample wells. The G6P concentrations in the test samples:

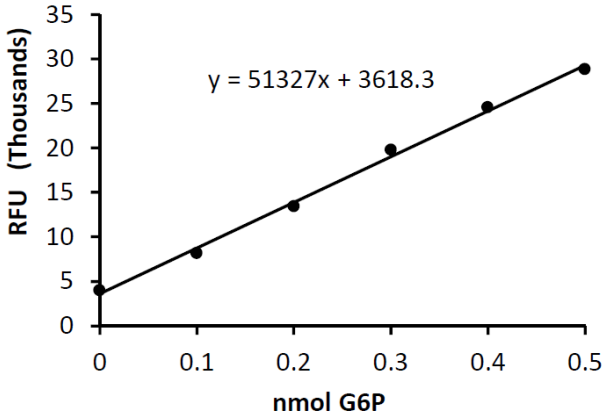
Concentration = Ay / Sv (pmol/µL; or nmol/mL; or µM)

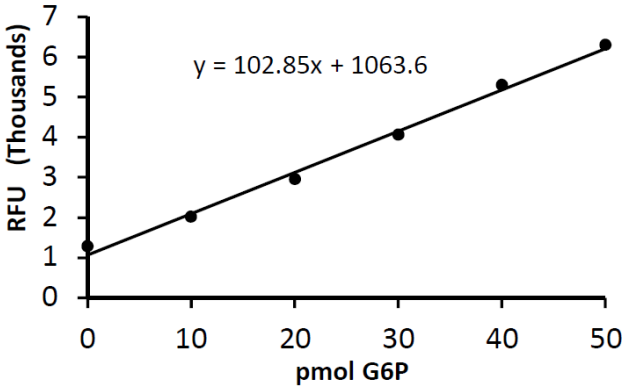
Where:

Ay is the amount of G6P (pmol) in your sample from the standard curve.

Sv is the sample volume (µL) added to the sample well.

Glucose-6-phosphate molecular weight: 260.14.





G6P Standard Curves (0-500 and 0-50 pmol range) generated using this kit protocol.

6. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Problem	Reason	Solution
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)